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o-PHTHALALDEHYDE STAINING OF COILED AND UNCOILED INTRAEPIDERMAL SWEAT DUCTS*

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ABSTRACT

(1) We have confirmed the view that the o-phthalaldehyde stain for sweat pores reflects the presence of trace amounts of both water and ammonia in the poral infundibulum.

(2) A technique is described for staining the intra-epidermal sweat duct *in situ* as well as *in vitro*.

(3) Straight uncoiled ducts have been observed in paronychial epidermis. Reduced coiling was commonly seen in ducts coursing through verrucae.

(4) The application of OPT to wet skin produces a diffuse darkening of the skin. This serves as a marker for stratum corneum turn-over and could be used to darken vitiliginous spots in deeply pigmented skin.

In 1967, Juhlin and Shelley (1) introduced a simple new method of visualizing the active sweat pore in the skin of man. In consisted of the topical application of a 5% solution of o-phthalaldehyde (OPT) in xylene. Within 2 to 3 minutes the eccrine sweat pores selectively stained black under suitable circumstances of minimal sweating. The black stain did not depend on the presence of visible sweat, but rather was an intrinsic poral stain which persisted for 5 to 8 days. It was attributed to the formation in the infundibulum of a black complex resulting from the interaction of the OPT with sweat ammonia deposited in the poral keratin. The stain has subsequently proven useful in both clinical (2) and experimental studies (3). The present study was undertaken to ascertain whether or not OPT could be used for staining the terminal intraepidermal eccrine sweat duct.

MATERIALS AND METHODS

o-Phthalaldehyde (Calbiochem, P. O. Box 54282, Los Angeles, California 90054) was employed directly in various organic solvents for the visualization of the sweat pore and the intraepidermal sweat duct in the skin of man (both in situ and in vitro). Epidermal shave biopsy specimens were also studied after 30–60 minutes immersion in OPT solutions. Very thin, free-hand vertical sections were cut, mounted in immersion oil and viewed under transillumination as well as by intense reflected light at magnification of from 1–300 power.

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RESULTS

o-Phthalaldehyde could be applied in a variety of ways to elicit staining of the eccrine sweat pores. The simplest maneuver was to rub the pure crystals on the skin surface. Less effective, but still capable of producing a stain, was exposure of the skin within a heated jar containing the OPT vapor. The most suitable method proved to be the use of OPT dissolved in a volatile anhydrous organic solvent in a concentration of 5%.

The following solvents were successfully employed: ethyl ether, xylene, acetone, tetrahydrofuran (Fig. 1) and chloroform (2 parts)—methanol (1 part).

The application of OPT dissolved in any organic vehicle produced diffuse black staining of the entire stratum corneum when wet. There was no selective poral staining, but this technique could be used as a darkening agent for leukodermatous areas in dark skinned individuals since the stain remained for 5 to 7 days. It was apparent that it could be used as an alternate marker for turnover time of the stratum corneum (4).

We were able to confirm the fact that selective OPT staining of the sweat pores requires the poral presence of trace amounts of both water and ammonia (1). Completely dry or anhidrotic sweat pores failed to stain, whereas as indicated the entire stratum corneum of wet skin stains black. Since we have found no individuals in whom ammonia was absent from the sweat, the decisive factor in staining proved to be the state of hydration of the sweat pore itself.

The awareness that OPT and ammonia from

sweat could unite in the presence of water to give a black non-fluorescent component indicated to us that it might be possible to stain the intra-epidermal sweat ducts. *In situ* experiments introducing the OPT solution by capillary pipette did result in selective staining of the intra-epidermal duct a dark black color. It was hard to reproduce regularly due to the fact that the average pore lumen is only 16μ (7) and hence the following technique was evolved.

Epidermal shave biopsy specimens were taken with a razor. On the palm or in working with hyperkeratotic lesions, these could be limited to the stratum corneum, but elsewhere the full epidermis was removed free hand (6). These specimens were immersed in 5% OPT in ethyl ether at room temperature for 30–60 minutes. Under a dissecting microscope very thin vertical sections were then made with a razor, cutting parallel to the ridge row of pores in the case of palmar skin. By viewing the sections laterally in immersion oil with magnification of from 7 to 30× it was possible to see the sweat ducts stained black (Fig. 2). Not all ducts stained and some stained only partially, i.e. at the free ends.

FIG. 1. o-Phthalaldehyde staining of palmar sweat pores of ridges of the fingertip. × 3½.

Random specimens from normal skin showed regular dextral coiling of the duct. This was most striking in the thick epidermis of the palm and sole. In normal skin, only the paronychial tissue showed ducts without coiling (Fig. 3). The reduction and absence of coiling was also clearly demonstrated in verrucae (Fig. 4). In psoriasis staining was strictly limited to the proximal sweat duct.

DISCUSSION

The techniques for staining the sweat duct and sweat pore have recently been reviewed (8). Although the time honored method of sweat pore staining is the iontophoresis of methylene blue (9), the OPT stain is simpler, requiring no apparatus. It does necessitate a slight activity of the sweat gland to provide moisture for the selective staining desired. The studies reported herein support the view that the ammonia of the sweat deposited in the infundibulum accounts for the black stain of the pore. Any of a wide variety of volatile organic solvents may be used for dissolving the OPT. We have not seen any adverse effects when this is used on normal skin. However, its use on tape-stripped permeable skin has been shown to have irritant effects (3). Interestingly the use of OPT on wet skin produces a diffuse pigmenting of the stratum corneum suitable for following turn over time of the stratum corneum or for darkening depigmented areas in dark skinned individuals. This deserves further study inasmuch as the stain remains for days and is not associated with irritation.

The visualization and staining of the sweat duct in the epidermis is more difficult. Takagi and Tagawa (10, 11, 12, 13) have studied this problem intensively with phase and regular microscopy. Others have made observations with photography under oil (14, 15). The ducts can be especially well visualized in the negro where the duct appears as a white thread against a dark background (16). Patients who have had atebrine show fluorescence of the terminal duct (17), and fluorescent dyes have been introduced into the duct for viewing (17). The capillary pipette has been

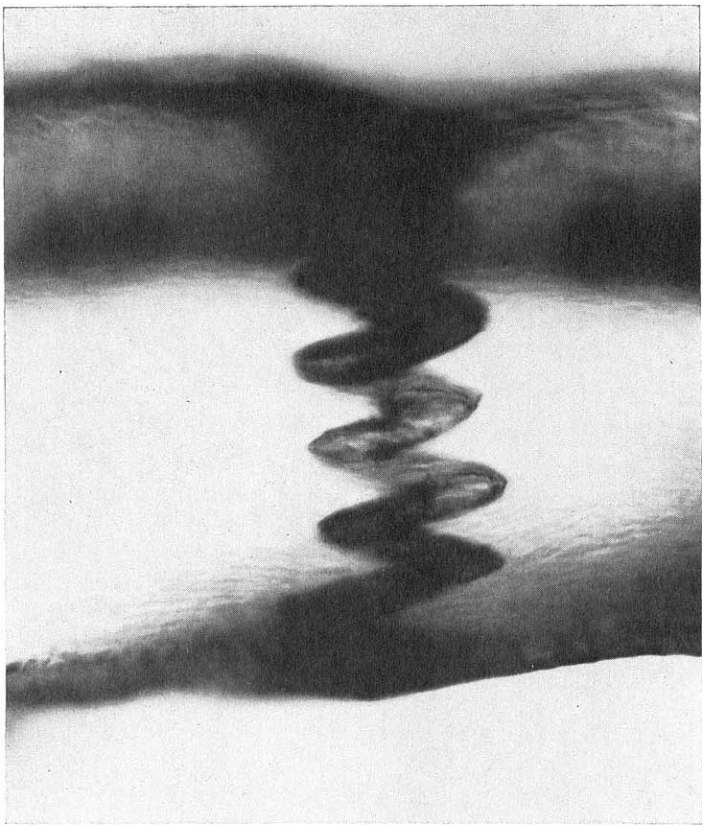

FIG. 2. Dextral helix of intra-epidermal sweat duct, in shave biopsy specimen of stratum corneum from palm stained with o-phthalaldehyde. Lateral view. × 300.

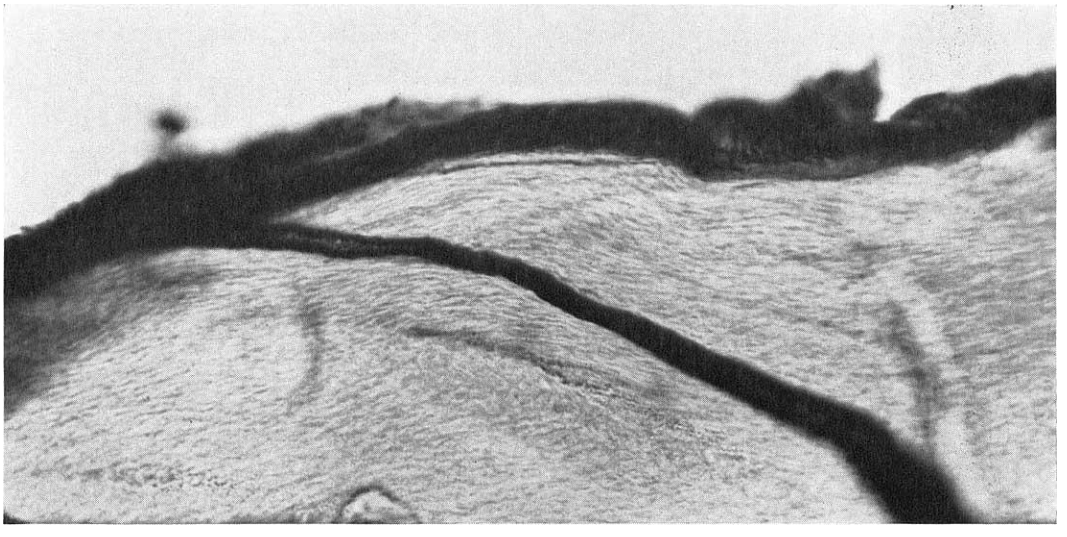

FIG. 3. Absence of coiling of intra-epidermal sweat duct in specimen of normal epidermis from paronychial area. o-Phthalaldehyde stain. × 220.

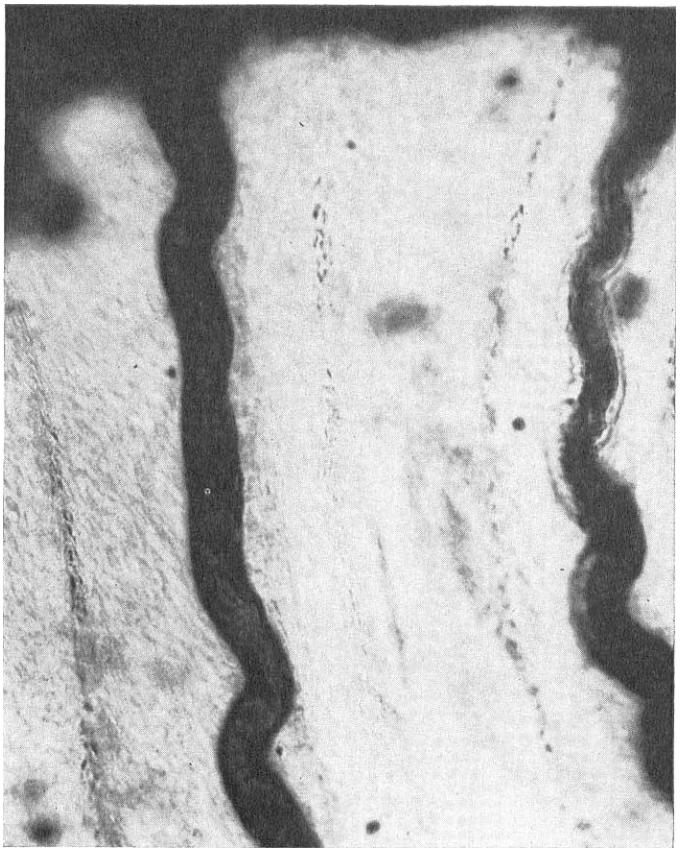

FIG. 4. Reduced coiling of intra-epidermal sweat duct coursing through the periphery of a verruca. o-Phthalaldehyde stain. × 155.

employed for sampling sweat as well as for introducing dye solutions directly into the straight intradermal duct of paronychia skin (18). Finally, the duct has been carefully studied in thick sections (19, 20), as well as in thin sections with histochemical as well as routine stains (16, 21). In our technique the majority of the ducts stained completely, but in some areas and in some subjects staining was incomplete, being limited to the free ends. Possible explanations for staining failure include the occluding effect of air bubbles and the presence of obstructive intra-luminal keratin deposits.

In all these reports the dramatic fact that the intra-epidermal duct coils dextrally (from left to right as viewed from the base) is repeatedly present (19, 22). The cause as well as the significance of this constant helical winding, irrespective of the side of the human body studied, is as yet obscure. It may be an intrinsic property of the duct per se since it winds even in the dermis (23) and it winds even when being reconstituted in regenerating epidermis following experimental injury (24). Nonetheless the screw-like ascent of the sweat duct in the epidermis is viewed by Wolf (25) as an indication of an analogous whorling growth of the epidermal tissue.

By contrast, the straight duct is anomalous occurring normally only in the paronychial tissue (13, 18). We have confirmed this and have also shown the presence of straight uncoiled ducts in verrucae. Finally, we have shown that the proximal end of the sweat duct in anhidrotic psoriatic skin stains, although the terminal duct and pore cannot be stained. This suggests an obstructive cause for the anhidrosis.

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